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Evaluation of Renal function tests in patients with cirrhosis of liver

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Abstract:
Cirrhosis is a diffuse hepatic process characterized by fibrosis and the conversion of normal hepatic architecture into structurally abnormal nodules. Cirrhosis represents the last histological picture for a large variety of liver diseases. The progression to cirrhosis is very uncertain and may occur over short time (weeks) to long time (many years). The liver and the renal systems are physiologically as well as pathologically interlinked. Hence a detailed study was undertaken to study the renal parameters in cirrhosis of liver,the focus is done to evaluate the diagnostic accuracy of plasma creatinine and creatinine based formulas in assessing renal functions in patients with chronic liver disease and to assess the diagnostic accuracy of cystatin C and cystatin C based equations in assessing renal functions in chronic liver disease patients and finally to proove whether etiology of chronic liver disease has an impact on renal function in these patients.

Key words: Creatinine, Cystatin C, Cirrhosis, Renal function

Introduction:

Renal dysfunction is a frequent complication in cirrhotics and this combination has got a significant morbidity and mortality. Both liver and kidney disease can occur simultaneously or a primary liver disease can lead to secondary abnormalities in kidney function [1].Decreased renal function in patients with cirrhosis is usually functional without any structural abnormality and occurs gradually over a period of time and has a negative impact on the prognosis of patients [2,3].The incidence of renal failure in advanced chronic liver disease ranges from 15% to 50% [4]. Patients with cirrhosis have circulatory dysfunction and diminished effective arterial volume leading to acute renal dysfunction in these patients. This acute kidney injury may be spontaneous or precipitated by infection, use of nephrotoxic drugs, blood loss or gastrointestinal fluid losses.The accurate assessment of renal function is these patients is providing to be difficult due to excessive dependence on the plasma creatinine whose levels are dependent on various factors like the severity of chronic liver disease, malnutrition and elevated bilirubin levels which interferes with the

estimation of serum creatinine. Conventional biomarkers like blood urea, plasma creatinine and urine markers are non specific and insensitive for the detecting an acute decrease in renal function in these patients. Newer biomarkers for detecting renal injury are being evaluated in various studies. These include both serum and urine biomarkers namely serum neutrophil gelatinase associated lipocalin (sNGAL) and cystatin C. Urinary biomarkers include kidney injury morecule (KIM-1), NGAL (u NGAL) and interleukin -18 (IL-18) [5].

It is imperative to detect the onset, severity and progression of renal dysfunction in cirrhotics as this has got a major impact on the survival of these patients and is one of the major risk condition affecting the prognosis in patients undergoing liver transplantation.Patients with pre transplant renal dysfunction are prone to develop complications than those without renal failure.This emphasizes the need for the earlier detection of renal dysfunction in cirrhotics for optimal management of these patients. This has prompted the evaluation of several biomarkers of renal injury like kidney injury molecule, serum and urine NGAL and cystatin C as markers of renal injury in these patients. Of these biomarkers Cystatin C has been studied the most in cirrhotics and it has been proved to be a superior marker to serum creatinine in this subgroup of patients.

Materials and Methods

Inclusion criteria: After they gave informed consent, 50 patient undergoing treatment for chronic liver disease as in-patients in the Medical College, participated in the study. The criteria for chronic liver disease is defined by Compatible clinical profile (History, Ascites with or without jaundice), Ultrasound evidence (reduced liver span/altered echo texture of liver), Biochemical profile (abnormal liver function tests or reversal of albumin- globulin ratio)
This analytical study was conducted in 50 eligible patients admitted for chronic liver disease with apparently normal renal function in the medical wards in our hospital from August 2011 to August 2013. All patients underwent a thorough clinical examination, including medical history.

Urinalysis including 24 hours urine volume and urine creatinine were done after stopping diuretics for three days and biochemical profile including liver function tests, viral markers for hepatitis B, renal function tests were done and results were noted.

After assessing thyroid status of the patient, serum cystatin was estimated with the same sample used for evaluation of plasma creatinine. Data about demographic variables, clinical features were collected using a proforma.

Ultrasound Abdomen was done in all the patients to assess liver size and echotexture, presence of splenomegaly, portal vein dilatation, presence of collaterals around spleen, presence of ascites and structural renal abnormalities.

Estimated Creatinine clearance was calculated using Cockcroft – Gault formula (CGF)

$$\frac{(140\text{-Age}) \times \text{Body weight in kilogram}}{\text{Serum Creatinine} \times 72}$$

If the patient is female the above value has to be multiplied by 0.85.
Measured creatinine clearance was calculated by the formula

$$\frac{\text{(Urine creatinine} \times \text{24 hours urine volume)}}{\text{Serum creatinine.}}$$

This value was divided by 1440 to get the GFR in ml/min
Estimated creatinine clearance using serum Cystatin was calculated using Hoek'sformula ,
GFR = -4.32 + (80.35 X 1/Cys C). Comparison of GFR obtained by the above methods was done.

Statistical Analysis

The continuous variable of two groups of study subjects were compared between attributes by student independent 't' test. More than two groups of variables were compared between the categories by ANOVA (Analysis of variances) and the significance between the two groups were tested by post coc test of Bonferrani. The category variables were compared by Chi square test. The above statistical procedures were performed by the statistical package IBM SPSS 20. The 'p' value less than 0.05 ($p<0.05$) was considered as significant.

Results

50 patients with chronic liver disease who fulfilled the inclusion criteria participated in the study. Among the study group the minimum age was 24 years and maximum age was 58 years. The mean age was 43.5 years. Of the 50 patients 40 were male and 10 were female.

Etiology of chronic liver disease:

Alcoholism was found to be the major cause of chronic liver disease followed by HbsAg. The other causes were Wilson's disease and Autoimmune. Causes could not be identified in 15 patients.

Table 1: Etiology of chronic liver disease

CAUSES	MALE	FEMALE	TOTAL
Alcohol	26	00	26
HbsAg	02	04	06
Wilson's	01	01	02
Autoimmune	00	01	01
Idiopathic	11	04	15
Total	**40**	**10**	**50**

Alcoholism was found to be the major cause of cirrhosis in males where as HbsAg positivity was found to be a major cause in females.

Table 2: Clinical characteristics of study participants:

Variable	Serum Bilirubin (mg/dl)	Serum Albumin (gm/dl)	PT (Sec)	Blood Urea (mg/dl)	Serum Creatinine (mg/dl)	Serum Cystatin (mg/L)	Urine value	Urine Creatinine
Mean	5.0	3.2	14.9	28.0	0.93	1.4	1446.8	59.1
SD	5.7	0.6	3.0	9.3	0.19	0.5	424.7	13.2
Min	1.0	1.8	12.0	15.0	0.6	0.73	400.0	43.0
Max	25.5	4.6	27.7	54.0	1.4	3.10	2300.0	102.0

The mean serum bilirubin was 5 mg/dl , serum albumin was 3.2 mg/dl, mean prothrombin time was 14.9 seconds, serum creatinine was 0.93 mg/dl, mean serum cystatin value was 1.4 mg/L. The blood urea levels were within normal limits and did not rise markedly even in patients with diminished GFR indicating that it is an unreliable marker for assessing renal function. The mean blood urea level was 28 mg/dl.

Table 3: Comparison of biochemical characteristics between cases with known causes and idiopathic

Variables	Causes				Difference b/w means	't'	Df	Significance
	Present n = 35		Absent n = 15					
	Mean	SD	Mean	SD				
Sr.bilirubin	6.0	6.4	2.1	1.1	3.9	2.241	48	P<0.05
Sr.albumin	3.2	0.6	3.1	0.5	0.1	0.429	48	P>0.05
PT (Sec)	15.1	3.3	14.4	2.1	0.7	0.822	48	P>0.05
Blood Urea	27.6	9.3	29.1	9.4	1.5	0.516	48	P>0.05
Sr. Creatinine	0.9	0.2	1.0	0.2	0.1	2.524	48	P<0.05
Sr. Cystatin	1.36	0.57	1.44	0.35	0.08	0.444	48	P>0.05
Urine volume of 24 hours	1458.9	448.5	1415.6	370.1	43.5	0.320	48	P>0.05
24 hr. U.Creatinine	59.7	15.0	59.6	7.3	2.1	0.516	48	P>0.05

It was found that there was no significant difference in serum albumin, PT (Sec), blood urea, serum cystatin, urine volume of 24 hours and urine creatinine of 24 hours (P>0.05). The serum bilirubin of subjects with known causes was 6.0±6.4 and subjects with idiopathic causes was 2.1±1.1 and the difference was statistically significant (P<0.05). Similarly the serum creatinine of subjects with known causes was 0.9±0.2 and subjects with idiopathic causes was 1.0±0.2 and the difference was statistically significant (P<0.05).

Grading of severity by child – pugh score:

Patients with score 5 to 6 fall under Grade A. Grade B includes scores from 7 to 9. Scores above 9 is Grade C. Of the 50 patients, sixteen patients belonged to Grade A, 21 came under Grade B and 13 were in Grade C. The mean score in Child Pugh A was 5.9, 8.0 in Child Pugh B and 10.7 in Child Pugh C.

Table 4: GFR BY COCKCROFT-GAULT METHOD:

Grading	GFR <50 ml/min	50-90 ml/min	>90 ml/min
Child Pugh A	0	10	06
Child Pugh B	04	10	07
Child Pugh C	02	07	04

According to CGF, none of the patients in Child Pugh A had GFR <50 ml/minute, and only two (15.38%) patients in Child Pugh C had GFR <50ml/min. The levels of serum creatinine across varying grades of Child Pugh scoring is as follows.

Table 5: Serum creatinine and child pugh score:

Child Pugh Score	Serum Creatinine (<1 mg/dl)	Sr. Creatinine (1-1.2 mg/dl)	Sr. Creatinine (>1.2 mg/dl)
A	10	06	0
B	11	09	01
C	06	06	01

Of the 16 subjects in Child Pugh A group 10 (62.5%) had a serum creatinine value of less than one mg/dl and none of them had creatinine levels above 1.2 mg/dl.

Of the 21 subjects in Child Pugh B, 20 had their serum creatinine levels within normal limits where as only one patient (4.76%) had serum creatinine above 1.2 mg/dl.

Of the 13 subjects with Child Pugh score C, six (46.13%) of them had their creatinine levels less than one and only one patient (7.69%) had his values more than 1.2 mg/dl.

Table 6: Serum cystatin c and child pugh score:

Child Pugh Score	S. CYSTATIN (<1 mg/L)	S. CYSTATIN (1-1.5 mg/L)	S. CYSTATIN (>1.5 mg/L)
A	9	5	2
B	3	10	08
C	1	04	08

Of 16 patients in Child Pugh A nine patients (56.25%) had serum cystatin levels less than one where as only one patient (7.69%) in Child Pugh C had cystatin levels less than one. Eight (61.53%) out of thirteen patients had serum cystatin level more than 1.5 in Child Pugh C. this shows that serum cystainlevels increase as the degree of liver dysfunction increases.

The mean cystatin level in Child Pugh A was 1.05 where as it was 1.47 in Child Pugh B and 1.66 in Child Pugh C.

Study subjects were classified into three groups based on their GFR calculated based on cystatin C. Group I had a GFR <50 ml/min. group II had GFR between 50-90 ml/min where as Group III had GFR above 90 ml/min.

Table 7: Serum creatinine and GFR

	S. Cr <1 mg/dl	S. Cr 1 to 1.2	S. Cr > 1.2 mg/dl
Group I	05	14	01
Group II	18	05	01
Group III	05	01	00

Of the 20 patients with GFR below 50 ml/min, 19 (95%) had serum creatinine values below 1.2 mg/dl and only one patient (5%) had serum creatinine values above 1.2 mg/dl indicating that even though patients have a reduced GFR the serum creatinine levels fail to raise above the normal limits correspondingly.

Table 8: Child pugh and GFR estimated by cystatin c

Child Pugh Score	GFR <50 ml/min	50-90 ml/min	>90 ml/min
A	02	10	04
B	08	11	02
C	10	03	00

Only two patients (12.5%) in Child Pugh A had GFR less than 50 ml/minute where as 10 (76%) patients out of thirteen in Child Pugh C had GFR <50 ml/minute. Four (25%) out of sixteen patients had GFR >90 ml/minute where as none of the patients in Child Pugh C had GFR >90 ml/minute when cystatin C is used.

GFR measured by timed 24 hour urine collections and 24 hour urinary creatinine across various grades of Child Pugh are as follows.

Table 9: GFR by measured creatinine clearance

Child Pugh Score	GFR <50 ml/min	50-90 ml/min	>90 ml/min
A	01	10	05
B	06	13	02
C	06	07	00

Only one patient (6.25%) in Child Pugh A had GFR <50 ml/minute where as six patients (46%) in Child Pugh C had GFR <50 ml/minute when creatinine clearance is measured.

The patients were grouped under three Categories. Group 1 included patients with GFR <50 ml/min according to hoek's formula Group 2 included those with GFR between 50 to 90 ml/min. Group 3 included those with GFR above 90 ml/min.

Table 10: Comparison of GFR by various methods

Methods	Group 1 (<50 ML/MIN)		Group II (50-90 ml/min)		Group III (>90 ml/min)	
Cockcroft Gault	07	(14%)	27	(54 %)	16	(32 %)
Hoek's Method	20	(40%)	24	(48 %)	06	(12 %)
Measured Creatinine Clearance	13	(26%)	30	(60 %)	07	(14 %)

On comparing GFR values obtained by the three methods, only seven (14%) out of fifty patients had GFR 50 ml/min by CGF, whereas 20(40%) was found to have GFR <50 ml/min when cystatin based formula is used. 13 (26%) subjects fell under group I when creatinine clearance was measured…

This shows that cystatin C based equations have better sensitivity in identifying small decline in glomerular filtration rate when compared to other methods.

Table 11:

Methods	Mean	SD	ANOVA 'F'	Sig.	Significantly differed Measures
CGF	76.3	20.7	7.060	P>0.001	CGF & Hoek
Hoak	54.4	21.1			CGF & Mea GFR
Measured GFR	65.3	22.9			Hoek and measured GFR

The above table compares the CGF, Hoek and measured GFR. The mean of CGF was 76 ± 20.7, which significantly differed with other methods namely Hoek (54±21.1) and measured GFR (65.3±22.9). P value was found to be <0.001 which is significant. The difference between Hoek procedure and measured GFR was satistically significant (p <0.05).

Renal function and etiology:

The study subjects were grouped into three categories according to their GFR. Group 1 with GFR less than 50 ml/min, Group 2 with GFR between 50 to 90 ml/min and those with GFR more than 90 ml/min fall under Group 3. The GFR was compared in subjects with chronic liver disease due to various etiologies.

Out of 26 alcoholics, eighteen subjects (69.23%) had GFR less than 50 ml/min, and the eight (34.78) had GFR in the range between 50-90 ml/min and none of them had GFR above 90 ml/min.

Chronic liver disease associated with Wilson's disease had GFR above 50 ml/min.

Serum albumin and renal function:

Our study group was divided into three categories based on their GFR and serum albumin level distribution in all the three groups were noted.

Table 12:

S. Albumin (mg/dl)	Group 1 (GFR<50ml/min)	Group 2 (GFR50-90 ml/min)	Group 3 (>90 ml/min)
<3.0	05	05	00
3.0 – 3.5	14	16	02
>3.5	01	01	06

The mean serum albumin level of the patients in three groups are

Group I – 3.02 gm/dl

Group II – 3.25 gm/dl

Group III – 3.51 gm/dl

Patients with higher GFR had marginally higher albumin levels. But it was not found to be statistically significant. (p>0.05).

Discussion

We analyzed the extent of renal dysfunction in 50 patients with chronic liver disease. Till now creatinine based equations for estimating glomerular filtration rate are the most commonly used methods. These have the advantage of being free of urine collection errors.

In our study, even in patients with glomerular filtration rate less than 50ml/min serum creatinine levels were found to be normal and failed to raise above the upper reference limits. The study by McAulay et al [6] also reported similar findings

that serum creatinine is a poor indicator of renal function in chronic liver disease and was found to be within normal limits, even when GFR has reduced significantly. The study by Caregaro et al [7] showed that serum creatinine had only 18.5% sensitivity in detecting reduced renal reserve in patients with cirrhosis at risk of renal dysfunction. Papadakis and Arieff [8] had also reported similar observations that use of serum creatinine solely as a marker of renal dysfunction is highly erroneous and leads to a spuriously good renal reserve in these patients.

This may be due to the fact that the steady state concentration of creatinine is low in cirrhotic. The various factors contributing to reduced serum creatinine levels include malnutrition and reduced muscle mass in these patients. Also the hepatic synthesis of creatine which is the precursor of creatinine is reduced by 40 to 50 % in these patients. Renal tubular secretion of creatinine also increases in these patients, which further decreases serum creatinine levels.

Our study also showed that creatinine clearance by Cockcroft – Gault formula overstimates GFR in chronic liver disease patients. This is in agreement with the study done by Skluzacek et al comparing Cockcroft Gault and MDRD based formulas with iodine 125-iothalamate clearance which has also shown that both CGF and MDRD are inaccurate and overestimate GFR. The study by McAulay et al reported similar observations and also showed that among the creatinine based equations MDRD formula is a better method for estimating GFR. This takes into account the patient's age, sex, race, serum cretinine, serum albumin and blood urea nitrogen levels.

This may be due to the fact that body weight is a numerator and serum creatinine is a denominator is Cockcroft Gault formula. Increased body weight due to ascites and edema with reduced serum creatinine contirbutes to the falsely elevated GFR by this method.

In our study serum cystatin C was found to raise as the renal function deteriorates indicating that it could be a reliable marker for renal dysfunction in decompensated chronic liver disease patients. 40% of the patients in our study had GFR <50 ml/min when cystatin based formulas were used compared to 14% when creatinine based Cockcroft Gault formula was used.

Our study is in accordance with that of the study by Poge [9] et al which was done to evaluate the diagnostic accuracy of cystatin based formulas namely Hoek and Larsson equations and it showed that there was a significant improvement in estimation of GFR with lower bais and higher precision than creatinine based formulas but none were as accurate as inulin clearance.

The study undertaken by Herget – Rosenthal [10] to identify the better method in detecting the occurrence of renal dysfunction between serum cystatin C and serum creatinine and, serum cystatin C detected ARF earlier than serum creatinine _by 1.5 ±6 days (Risk criteria) and 1.2±0.9 days by injury criteria.

Our study is in agreement with the Rocco Orlando et al [11]. Their study showed that serum cystatin C had a good diagnostic sensitivity (88%) when compared to serum creatinine (23%) to detect renal dysfunction. In cirrhotic patients.

Newman et al [12] reported similar results that not only serum cystatin C is a better marker than serum creatinine and that it is more sensitive to detect smaller changes in GFR. In our study too serum cystatin proved to be better than other methods.

In a meta analyses by Vikas Dharnika et al [13], Cystatin C has got a greater correlation coefficient than creatinine and the correlation of GFR with the reciprocal of Cystatin C increases as the renal function deteriorates.

In our study measured creatinine clearance by timed urine collections was better than serum creatinine and estimated GFR by Cockcroft – Gault method but less accurate than cystatin based formulas.

The study by Proulx et al [13] showed that though Inulin clearance is the most accurate method for estimating renal function, it is practically impossible in resource limited settings and though calculated GFR by timed urine collection methods overestimates true GFR it is preferable to that of Cockcroft Gault formula.

The overestimation of true GFR may be due to the fact that there is increased tubular secretion in the setting of lower glomerular filtration leading to falsely high values of glomerular filtration rate.

The study by Papadakis and Arrief and another study by Proulx et al [14] have supported this observation by concluding that calculated creatinine clearance may be an aid to true GFR in the absence of Inulin clearance. In our study there was no statistically significant correlation between the levels of serum albumin and renal function.

This is in agreement with a study by Hampel et al [15] which also had reported that serum albumin levels did not correlate with degree of renal

dysfunction and it was not considered to be a significant risk factor for the development of renal dysfunction.

Our study has also shown that patients with alcoholic cirrhosis had adverse renal funciton compared to those with HbsAg positivity. 69.23% of alcoholics had GFR below 50 m/min where as only 33.33% of HbsAg positive individuals had GFR <50 ml/min.

In our study blood urea levels were found to be normal even when GFR was grossly reduced. This may be due to their reduced synthesis in patients with decreased hepatic function. Also they may falsely increase when there is gastrointestinal blood loss. Hence they are unreliable for assessing renal function in cirrhotics.

Conclusion

In patients with chronic liver disease, plasma creatinine alone is a poor marker for detecting renal dysfunction. Creatinine based Cockcroft – Gault formula grossly overestimates renal function. Serum Cystatin C and cystatin C based formulas for estimating GFR is a better marker of renal dysfunction compared to serum creatinine and hence should be used for assessing renal function for making necessary dose adjustments in these patients. Measured creatinine clearance though not accurate as cystatin C based formulas is better than Cockcroft Gault method. Alcoholics develop severe renal dysfunction compared to patients with cirrhosis due to other causes.

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